

Abstract

The present invention relates to methods of producing a heterologous biological substance, comprising: (a) cultivating a mutant of a parent *Aspergillus niger* strain in a medium 5 suitable for the production of the heterologous biological substance, wherein (i) the mutant strain comprises a first nucleotide sequence encoding the heterologous biological substance and one or more second nucleotide sequences comprising a modification of *glaA* and at least one of the genes selected from the group consisting of *asa*, *amyA*, *amyB*, *prtT*, and *oah*, and (ii) the mutant strain is deficient in the production of glucoamylase and at least one enzyme 10 selected from the group consisting of acid stable alpha-amylase, neutral alpha-amylase A, and neutral alpha-amylase B, protease, and oxalic acid hydrolase compared to the parent *Aspergillus niger* strain when cultivated under identical conditions; and (b) recovering the heterologous biological substance from the cultivation medium. The present invention also relates to enzyme-deficient mutants of *Aspergillus niger* strains and methods for producing such 15 mutants.